IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	Art Unit: 1638
Heifetz *et al.*	Examiner: A. Mehta
Appl. No. 09/309,038	Atty Docket: A-30496B
Filed: May 10, 1999	
For: Regulation of Gene Expression	

DECLARATION OF IAN EVANS UNDER 37 CFR §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, IAN EVANS, declare as follows:

1. I am a Senior Scientist with Syngenta Limited (formerly Zeneca Limited). My credentials are set out in my Curriculum Vitae, a copy of which is attached to this Declaration as Exhibit A.

2. On the basis of my qualifications set forth in Exhibit A, I submit that I am qualified to speak of the skill and knowledge of those skilled in the arts pertaining to the subject matter claimed in the above-identified application, particularly with respect to methods for conferring resistance or tolerance to a virus upon a cell by introducing into a cell and plant a sense and antisense RNA fragment of a viral genome and DNA constructs as described and claimed in the above-identified application.

3. I am familiar with the above-identified application and the issues raised in the Office Action dated August 12, 2003, and I make this Declaration to address the issue that the specification of the above-identified application enables the methods and DNA constructs as claimed.

4. It is my opinion that those skilled in the arts pertaining to the subject matter claimed in the above-identified application, given the description and teachings in the above-identified application and the knowledge in the art, would be able to prepare DNA constructs comprising a first and second

DNA sequence capable of expressing in a cell a sense and antisense RNA fragment of a viral genome or portion thereof and further, wherein the DNA sequences are operably linked to one or more promoters. Also, one skilled in the art would be able to follow the methods described and taught in the instant specification to introduce into a cell sense and antisense RNA fragments of a viral genome or portion thereof, wherein the expression of the viral genome or portion thereof of a potyvirus, tospovirus or cucomovirus in the cell is reduced. The results of experiments conducted by the groups of Dr Marcel Prins (Wageningen, NL) and Dr Craig Sandlin (Syngenta) form the basis for this opinion. These experiments are described below.

Tospovirus Experiments

5. Vectors were constructed to produce multivirus resistance to the tospoviruses, TSWV, GRSV, TCSV and WSMV by Etienne Bucher and Marcel Prins at Wageningen University (an earlier student M P van der Heijden also contributed significantly to making the constructs). A fragment of each virus of about 150 bp was inserted in a concatemer fashion to create an RNA that will form a dsRNA. The construct utilized the "N gene" (nucleoprotein gene) virus fragments to combat four tospoviruses, which are related to each other but different. As shown in the diagram, the concatemer virus fragments were separated by a fragment of DNA encoding the Arabidopsis actin intron. See Exhibit B. The vector construct IRB had the fragments in opposite directions and the IRN vector had the fragments pointing inwards towards the intron. See Exhibit B. The vectors were transformed into *Nicotiana benthamiana* plants using standard Agrobacterium transformation procedures.

6. Analysis of the transgenic plant lines to virus infection showed that 13 of the 17 IRB lines tested so far were resistant against all four viruses (see Table in Exhibit B). A small problem with the TCSV inoculum was encountered because it was contaminated with another virus which could not be identified. All lines tested for TCSV showed a virus-susceptible phenotype; however, when the plants were tested by ELISA and it was found that only infected control plants actually contained TCSV and not the transgenic lines. This indicated that the transgenic plants are indeed TCSV resistant.

Cucomovirus and Potyvirus Experiments

7. DNA constructs were made as described in the present specification on page 15, second paragraph and page 18, second paragraph. A schematic drawing of the resulting DNA construct (pZU684) is attached as Exhibit C. Vector pZU684 (4' in 1') was constructed to provide multivirus

resistance (MVR) to four major cucurbit virus groups (all ssRNA viruses). The Cucomovirus was CMV (Cucumber Mosaic virus) and three potyviruses were (WMV2, ZYMV and PRSV; Watermelon mosaic virus 2, Zucchini yellow mosaic virus, Papaya Ringspot virus) An unmodified virus sequence of approximately 600 nucleotide region of each virus was used. A partial coat protein cistron and the 3' non-translated region was used from the potyviruses and the 3' part of RNA2 was used from CMV. An intron separated the antisense and sense multivirus regions. The data for various lines of transgenic melon plants is shown in Exhibit C. Line 684-42 showed good resistance to ZYMV, PRSV and CMV with testing for WMV underway. Line 684-12 also showed some resistance.

8. The above tests demonstrate clearly that the DNA constructs as described in the specification and claimed in the above-identified application when introduced into a cell or plant using the methods as described and claimed are capable of altering or reducing the expression of a viral genome or portion thereof in the cell or plant as described and claimed in the above-identified application.

9. Thus, in my opinion, the aforementioned experimental results demonstrate that the methods, cells, plants and DNA constructs of the claimed invention are described in such a way as to enable those skilled in this art to make and/or use the invention.

10. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true. Further, these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Subscribed to on the following date:

11 February ,2004 IJ Evans

IAN EVANS